NEW NICOTINIC AGONISTS, ANTAGONISTS, AND ION CHANNEL BLOCKERS TO PROBE MULTIPLE SITES OF NICOTINE'S ACTION IN BRAIN.

L.G. ABOOD, S. BANERJEE, AND J. PUNZI

DEPT. OF PHARMACOLOGY, U. OF ROCHESTER MEDICAL CENTER, ROCHESTER, NY 14642

With the use of (-)-^{3}H-nicotine and, particularly, ^{3}H-1-methyl-2-(3-pyridyl)-azetidine, a more potent nicotine analogue, Scatchard analyses have revealed the existence of two distinct receptor binding sites with K_d values of 0.7 pM and 2 nM and B_{max} values of 3 and 25 fmoles mg protein; wheras, with either ^{3}H-acetylcholine or ^{3}H-methylcarbamylcholine, a pure nicotinic agonist, only the lower affinity binding site is observed. In an effort to examine the mechanism whereby mecamylamine and other ion channel blockers antagonize the central actions of nicotine, binding studies were performed on rat brain membranes with ^{3}H-mecamylamine. Scatchard analysis revealed the presence of two sites with K_d values of 96nM and I um and B_{max} values of 7 and 30 pmoles/ mg respectively. With a series of mecamylamine and pempidine analogues a good correlation was observed between their affinity for the ^{3}H-mecamylamine binding site and their ability to prevent nicotine-induced seizures in mice and prostration produced by intraventricular administration of nicotine to rats. The ^{3}H-mecamylamine site was inhibited by mM concentrations of monovalent cations and sub- mM concentraions of Ca and other cations; and it was completely inhibited after exposure to high T, trypsin, and detergents. Although mecamylamine does not compete for ^{3}H-nicotine binding, nicotine and its analogues exhibit a high affinity for the ^{3}H-mecamylamine binding site The findings suggest that nicotine may be acting both at the nicotinic recognition site and the associated ionic channel. Structure-activity studies with various carbamate esters of choline and other alkylaminoalkyl and heterocyclic amino alcohols reveal that the addition of alkyl substituents on the carbamyl N of choline and other amino alcohols abolishes muscarinic cholinergic properties while enhacing nicotinic properties. Replacement of the methyl group of methylcarbamylcholine by aromatic, heterocyclic, and aliphatic substiutents converts the compounds into pure nicotinic antagonists. Examples of effective nicotinic antagonists are phenylcarbamylcholine, quinuclidinylmethyl carbamate, and benzoylcholine. The specificity of methylcarbamylcholine and the substituted carbamte esters of choline for the nicotinic recognition site was established by demonstrating their very low affinity for the muscarinic cholingergic site (as measured by ^{3}H-quinuclidinylbenzilate binding) and the inability of the agonists to stimulate phosphoinositide (PI) turnover or the antagonists to inhibit carbamylcholine-stimulated PI turnover.